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ANTIBODY TO THE RNA-DEPENDENT DNA POLYMERASE OF HTLV-III: CHARACTERIZATION AND CLINICAL ASSOCIATIONS

ANNUAL REPORT

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Epitopes related to the HIV-1 polymerase important both to the identification of in vitro cellular responses correlating with disease progression, and as targets for immune intervention in HIV infection were explored. Additional serum samples from individuals at various WRAIR stages of HIV infection were examined. Synthetic peptides were prepared from nucleotide sequences of HIV pol regions homologous to the catalytic sites of other mammalian polymerases. A statistically significant but imperfect correlation was found between the presence of antibodies capable of blocking the catalytic activity of RT and anti-peptide II (~~VLPQGWKGSP~~; a.a. 158-167), and IV (PENPYNTPVFAIKK; a.a. 219-232) reactivity.

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Problem under study: The identification and characterization of anti-HIV reverse transcriptase antibodies in the sera of HIV seropositive individuals, and correlation of their levels with clinical status and defects in immune function.

In my Annual Report of 1987 we described the identification of a series of purified IgGs from HIV seropositive individuals capable of blocking the catalytic activity of HIV-associated reverse transcriptase. The specificity of these antibodies, lack of cross-reactivity with mammalian and prokaryotic DNA polymerases, and correlation with clinical health was described. This work culminated in one publication:

Laurence J, Saunders A, Kulkosky J. 1987. Characterization and clinical association of antibody inhibitory to HIV reverse transcriptase activity. SCIENCE 235:1501-4.

Summary of progress in 1988

1. Over the past year we have greatly expanded these studies. First, we have prepared bulk quantities of these anti-polymerase IgGs, and these are on deposit with the NIH as:

AIDS Research and Reference Reagents Program
AIDS Program
NIAID, NIH
"Antibody to HIV reverse transcriptase"
Catalog number: 187.

We have enclosed a copy of the catalog page, and a description of its preparation. Dr. Susan Stern, who administrates that program, has said she has already received 40 requests for this material.

2. We have received 78 (38 HIV seropositive) serum samples from Dr. Zvi Bentwich, Kaplan Hospital, Rehovot, Israel together with clinical and immunologic data. We have correlated serum p24 antigen levels with anti-RT activity and related these to clinical course, as described below.

3. We have recently received 150 coded serum samples from Dr. Robert Redfield at WRAIR, all of which are from HIV seropositive individuals at various clinical stages of infection. Over the next year we plan to investigate these samples in an attempt to design a synthetic peptide-based ELISA system for detection of anti-RT antibodies. This would enable us to avoid the laborious procedures needed for characterization of anti-RT activity by enzyme purification and enzyme inhibition.

4. In addition to these phenomonologic studies, we are looking at the mechanisms of early and latent HIV replication as potential

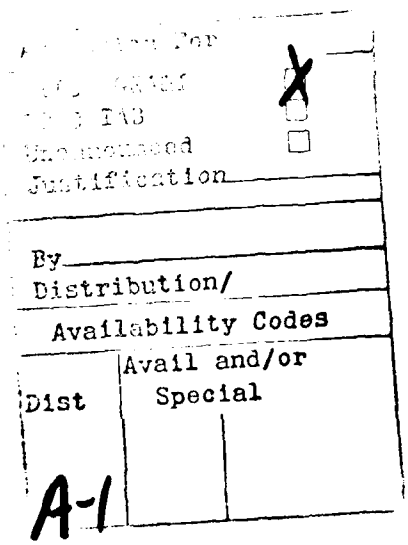
models for asymptomatic infection in man. This has resulted in three papers accepted for publication, all crediting the U.S. Army Medical Research Acquisition Activity contract. Copies of these manuscripts are enclosed, and summarized below.

Recent manuscripts acknowledging WRAIR support

1. Laurence J, Friedman SM, Chartash EK, Crow MK, Posnett DN. 1989. Human immunodeficiency virus infection of helper T cell clones: early proliferative defects despite intact antigen-specific recognition and interleukin-4 secretion. *J CLIN INVEST*, 83:1843-1848.

2. Laurence J, Sellers, MB, Sikder SK. 1989. Effect of glucocorticoids on chronic human immunodeficiency virus (HIV) infection and HIV promoter-mediated tyranscription. BLOOD, 74:291-297.

3. Laurence J, Sikder SK, Jhaveri S, Salmon JE. Phorbol ester-mediated induction of HIV-1 from a chronically infected promonocyte clone: blockade by protein kinase inhibitors and relationship to tat-directed trans-activation. BIOCHEM BIOPHYS RES COMMUN, in press.



GOAL 1. Bulk preparation of anti-RT antibodies. As indicated in the attached catalog page, we have prepared purified IgGs for the AIDS Program reagent bank established by the NIH. Immunoglobulin G (IgG) was isolated from each serum by ammonium sulfate fractionation and Zeta-Chrom 60 filter separation (CUNO Lab Products, Meriden, CT).

ANTIBODIES: POLYCLONAL AND MONOCLONAL

Antibody to HIV Reverse Transcriptase **Catalog number: 187**

Host: *Human.* **Isotype:** *Various polyclonal IgG.* **Titer:** *Concentration is 1 mg/ml.* **Special characteristics:** *IgG was isolated from serum of individuals exposed to HIV, using salt precipitation and ion-exchange. Protein is in PBS with no azide, sterile, frozen and shipped on dry ice.* **Source:** *Dr. Jeffrey Laurence.* **References:** *Science 235:1501, 1987.*

Antiserum to gp160-HTLVIIIB (HT3) **Catalog number: 188**

Host: *Goat.* **Isotype:** *Various polyclonal antibodies.* **Titer:** *1:3,000-15,000 obtained by endpoint dilution with ELISA against pre-immune serum from same animal.* **Special characteristics:** *Antiserum is specific for the entire sequence of gp160 HTLVIIIB (HB10) derived from baculovirus.* **Source:** *Dr. John McGowan, NIAID; produced under contract by Repligen.* **References:** *Proc. Natl. Acad. Sci. USA 84:69, 1987. NOTE: Available only as a single shipment of 200 µl per laboratory.*

Antiserum to gp160-HTLVIIIB and gp160-HTLVIIIRF **Catalog number: 191**

Host: *Goat.* **Isotype:** *Various polyclonal antibodies.* **Titer:** *1:3,000-15,000 obtained by endpoint dilution with ELISA against pre-immune serum from same animal.* **Special characteristics:** *Antiserum was obtained by co-inoculation of gp160 from HTLVIIIB and HTLVIIIRF and reacts with both proteins, which are derived from baculovirus.* **Source:** *Dr. John McGowan, NIAID; produced under contract by Repligen.* **References:** *Proc. Natl. Acad. Sci. USA 84:69, 1987. NOTE: Available only as a single shipment of 200 µl per laboratory.*

Antiserum to gp160-HTLVIIIB/HTLVIIIRF Hybrid (HT6) **Catalog number: 190**

Host: *Goat.* **Isotype:** *Various polyclonal antibodies.* **Titer:** *1:3,000-15,000 obtained by endpoint dilution with ELISA against pre-immune serum from same animal.* **Special characteristics:** *Antiserum is specific for gp160 of HTLVIIIB containing a substituted PB1 domain from HTLVIIIRF (amino acid residues 295-474). Protein is derived from baculovirus.* **Source:** *Dr. John McGowan, NIAID; produced under contract by Repligen.* **References:** *Proc. Natl. Acad. Sci. USA 84:69, 1987. NOTE: Available only as a single shipment of 200 µl per laboratory.*

Goal 2. Expand the numbers of clinical specimens evaluated for anti-RT activity. The following sera were obtained from Dr. Zvi Bentwich, originally as coded samples

Group I: n = 24
HIV seropositive
Clinical data: CD4+ T cells < 400/mm3
lymphadenopathy or other clinical manifestations of HIV infection

Group II: n = 14
HIV seropositive
Clinical data: CD4+ T cells $\geq$ 400/mm3
asymptomatic

Group III: n = 13
HIV seronegative
Clinical data: "non-HIV immune defects"

Group IV n = 12
HIV seronegative
Clinical data: HIV at risk group, normal immune function

Group V n = 15
HIV seronegative
Clinical data: non-HIV risk group, normal immune function

These sera were evaluated for antibody against the HIV RT catalytic activity, as described by the assays in our last annual report, as well as circulating p24 antigen by ELISA (Abbott Labs, Chicago, IL). None of the HIV seronegative samples expressed either p24 antigen or anti-RT activity.

As shown in Table I, 6/24 (25.0%) of Group I samples were positive for p24 antigenemia, while 1/14 (7.1%) of Group II samples were as well. In contrast, anti-RT antibodies were noted in 2/24 (8.3%) of Group I, but 8/14 (57.1%) of Group II samples. These data support our original observation of association of such antibodies with improved clinical status. It also emphasizes the fact that while p24 antigenemia tends to be associated with advancing clinical stage of HIV infection, this correlation is far from perfect. Combination of anti-RT antibodies with other parameters such as absolute CD4+ T cell count, serum p24 levels, etc., may assist in forming an improved prognosdtic indication for state of HIV infection.

Table I. Correlation of p24 antigenemia and anti-RT antibodies with degree of clinical or immune deficit in HIV infection.

GROUP I: HIV seropositive, symptomatic			GROUP II: HIV seropositive, asymptomatic, CD4+ T cells >400/mm3		
Sample code	p24 antigen (pg/ml)	anti-RT	Sample	p24 antigen (pg/ml)	anti-RT
778	1212	-	99	0	-
840	0	-	662	0	+
261	0	-	272	14	-
713	0	-	91	0	+
589	0	+	365	0	+
288	0	+	661	0	+
634	0	-	222	0	+
842	0	-	759	0	+
241	240	-	641	0	-
482	0	-	67	0	-
432	162	-	580	0	-
878	>8000	-	765	45	-
830	0	-	169	0	-
449	0	-	254	0	-
668	40	-			
320	0	-			
289	0	-			
815	0	-			
930	5152	-			
707	0	-			
966	0	-			
626	0	-			
469	0	-			
237	0	-			
% positive	25.0	8.3		7.1	57.1

GOAL 3. It appears unlikely that anti-RT antibodies themselves are responsible for the improved clinical status of individuals with high titer activity against the catalytic activity of HIV polymerase. It has been suggested that RT products may serve as targets for cytotoxic T cells, or that individuals with these antibodies have low levels of replicating HIV, with most cells infected in a chronic or latent state. To investigate this latter possibility, we have established a system of non-transformed human CD4+ T cell clones, and transformed human promonocytic cell lines which are chronically infected with HIV. In a series of three papers here appended we demonstrate how these cells can be used to investigate conversion of a latent state to active viral replication. We have examined the dependence of this conversion on protein kinase C, and have investigated a series of potential viral inducers and suppressors.

We plan to use this system as a model to investigate T cells and macrophages isolated from individuals with high titer anti-RT antibodies vs. cells from those that have lost such reactivity.

FUTURE PLANS

GOAL 4 : Improve the method of detection of anti-RT antibodies. The method by which these antibodies are presently sought is laborious and requires intact HIV reverse transcriptase capable of using a synthetic template. We are attempting to identify small peptides derived from the HIV pol sequence which might be used in an ELISA-based system for the recognition of these antibodies.This is being pursued by the following methods

Peptide selection. Using published nucleotide and amino acid sequences, we selected five linear peptides on the basis of homologies with putative catalytic sites of murine leukemia virus RNA directed DNA polymerase activity. These are listed in Table II. The peptides, ranging in lengthy from 8 to 155 amino acids, were synthesized at The New York Blood Center by the solid-phase method using an automated peptide synthesizer (Applied Biosystems, Foster City, CA). Peptide resins were cleaved by hydrogen fluoride, extracted, and analyzed for purity by HPLC.

Table II. PEPTIDES FOR ANTI-REVERSE TRANSCRIPTASE ELISA

Code	Sequence	Position	No.	Activity	Reference
I	LDVGDAYF	109 - 116	8	NTP binding site	Larder, Nature 327:716
II	VLPQGWKGSP	148 - 157	10	conserved among retroviruses	Larder
III	IQKLVGKLNW	257 - 266	10	conserved	Larder
IV	PENPYNTPVFAIKK	219 - 232	14	conserved	Johnson, PNAS 83:7648
V	DSRNPLWKGPAKLLW	496-509	15	serologic response	Warren, Poster 2216, IVth Int'l AIDS Conf.and Johnson

ELISA. Peptide solutions of 10mg/ml in PBS were air drived overnight at 37oC in polyvinyl, 96-well microtiter plates to yield 1-5 ug/well. Nonspecific binding sites were blocked with BSA. After a 6 h incubation at 25oC and a 16 h incubation at 4oC, serum samples were added at 1:100, 1:200 and 1:400 dilutions. After 2 h at 25oC the wells were washed 10-20 times with 0.2% Tween 20 in PBS, aftyer which affinity-purified, alkaline phosphatase conjugated goat antibody to human IgG was added. After 2 h the plates were washed, and substrate solution was added. After a 30 min. incubation the reaction was stopped by adding 1N H_2SO_4, and the optical densities were analyzed on an automated ELISA scanner. Seropositivity was defined as any value greater than twice the negative controls.

SAMPLES. The samples analyzed include 40 sera obtained from our original (1987) study, as well as 150 samples obtained from Dr. Redfield. Partial results indicate a correlation between anti-RT enzymatic inhibitory activity and recognition of Peptides II and IV; these data will be discussed at our annual meeting on March 27th.

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